REMARKS

Claims 37-55 are pending. Claims 37, 41, 42, 50, and 54 have been amended. Amendments to the claims are indicated in the section entitled "Version With Markings to Show Changes Made" and a list of the now pending claims is provided in the section entitled "Appendix of Pending Claims."

Claim Rejections - 35 USC §112, First Paragraph

Claims 37-55 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking sufficient written description to show possession of the claimed invention. Specifically, the Examiner argues that the specification and claims do not indicate what distinguishing attributes are shared by members of the genus recited as "isolated p42 protein" or "isolated p42 fragment. " The genus is said to be "highly variant because a significant number of structural differences between genus members is permitted." The Examiner further asserts that the specification fails to disclose a function shared by the species disclosed as SEQ.ID NO:2-5, and that one of ordinary skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species representative of the genus.

Applicant respectfully traverses, and requests reconsideration of the claims in light of the present amendment and remarks. Firstly, Applicant notes that each of base claims 37 and 50 has now been amended to include the additional structural recitation that the p42 polypeptide comprises at least a portion of the 42 kDa C-terminal processing fragment of the major merozoite surface protein gp195 from a *Plasmodium falciparum* isolate, and which shares at least one antigenic epitope with a polypeptide according to any one of SEQ.ID NO. 2-5. Support for the amendment can be found throughout the specification and in particular at, *e.g.*, page 3, line 9, page 7, lines 15-26, page 8, line 1 through page 9, line 3, page 12, lines 6-35 and also in Example 4.

Further, Applicant respectfully disagrees with the Examiner's suggestion that the

specification fails to disclose a representative number of species of such p42 polypeptides. The specification discloses corresponding amino acid sequence structures for p42 polypeptides from 4 different isolates of *Plasmodium falciparum*, therein designated FUP, MAD, WEL and K1. Each of the disclosed p42 polypeptides are described as being processing fragments of a gp195 surface glycoprotein from different *Plasmodium falciparum* isolates. The exact coordinates for the amino acid residues relative to the gp195 allele of each isolate are provided. More specifically, the p42 polypeptide corresponds to amino acid residues 1333-1726 of the FUP isolate, residues 1308-1701 of the MAD isolate, residues 1264 to 1640 of the WEL isolate and residues 1255 to 1631 of the K1 isolate. It is clear from Figure 6 that the corresponding amino acid sequences of p42 polypeptides are readily determined by aligning the C-terminal sequences of the various gp195 isolates in a manner that maximizes the sequence identity between them. Such methodology is commonly used in the art to identify corresponding amino acid sequences between allelic variants within a genus.

Moreover, based on the prior art and the specification, one of ordinary skill in the art would conclude that the genus of *Plasmodium falciparum* gp195 alleles (and hence corresponding p42 polypeptides) is not as large as the Examiner seems to envision. For example Kang and Long , *Mol. and Biochem. Parasit.* 73 (1995) 103-110 teaches that after analysis of the C-terminus of the merozite surface protein (gp195) from 15 different isolates of *Plasmodium falciparum* from Africa, Asian and Latin America, only a few nucleotide changes were found leading to amino acid alterations at only four positions out of 102 residues. The same reference concludes that there are only two gp195 alleles. Applicant's specification likewise identifies these two alleles in the p42 polypeptides represented on the one hand, by FUP and MAD that have few amino acid variations between them and on the other hand, by WEL and K1 isolates also having few amino acid differences between them As mentioned above, Applicant's specification provides the corresponding sequence coordinates between the p42 proteins for the two allele types. In addition, Example 11 discloses that Southern blot hybridization using probes for the two alleles revealed that FUP and three other isolates designated Pf857, FVO and Hond-1 were characterized as having either the MAD allele or the K1 allele. In the same vein, Ferreira et al, *Gene* 304 (2003) 65-75 recently analyzed

gp195 alleles in 60 isolates from Brazil and 37 from Vietnam, and mentions that with respect to block 17 (a 19 kDa region at the C-terminus) there were only five single nucleotide polymorphisms. Accordingly, there is not as much structural variation in the C-terminus regions of gp195 alleles as the Examiner seems to envision.

The specification further teaches that the C-terminus may preferably be truncated to remove the hydrophobic tail sequences. After the truncation, the remaining polypeptides correspond to sequences 1333 –1705 of the FUP isolate, 1308-1680 of the MAD isolate, 1264-1619 of the WEL isolate and 1255-1610 of the KI isolate. Considering the 4 isolates and the 4 truncated versions thereof, there are 8 disclosed species of p42 polypeptides sharing common features. Each of these are between 356 to 394 amino acids in length and each are defined by corresponding amino acid residues positions readily identified by positional alignment with one another. Therefore, the specification provides detailed structural information about 4 different species isolates of p42 polypeptides obtained from the four different gp195 proteins from *Plasmodium falciparum*. In addition, 4 truncated variants of these species isolates are precisely disclosed in the specification.

Based on the above, one of ordinary skill in the art would conclude that the amount of structural variation in the C-terminus of gp195 alleles represented by the p42 polypeptides disclosed by Applicant is not overly broad and that Applicant has disclosed adequate structural features of a representative number of species thereof.

In addition to structural features shared in common, the p42 polypeptides share functional characteristics, especially when combined with an adjuvant selected from QS-21 and ISA51 or combinations thereof. The p42 polypeptides each elicit antibodies that are functionally effective against various *Plasmodium falciparum* isolates. The antibodies disclosed and described in the specification are shown to be extensively cross-reactive with different parasite strains and to strongly or completely inhibit the growth of both heterologous and homologous *Plasmodium falciparum* parasites.

Applicant directs the Examiner's attention to Example 9 (pages 42-43) of the specification demonstrating that rabbits intramuscularly immunized with BVp42 (*e.g.*, a variant form of the natural gp195 p42 processing fragment) produce serum antibodies that

bind to both recombinant BVp42 (demonstrating immunogenicity) and the native gp195 (demonstrating cross-reactivity), as shown by ELISA (*e.g.*, vinyl plates coated with recombinant p42 and parasite gp195), parasitic indirect immunofluorescence (*e.g.*, acetone-fixed blood smears of schizonts and merozoites), and immunoblotting with parasite gp195.

BVp42 is demonstrated to be highly immunogenic (*see e.g.*, Table 5) and elicits antibody titers comparable to the antibody titers elicited from rabbits which have been immunized with the purified, parasite gp195. High ELISA titers were obtained late in the quaternary response for both BVp42 and purified parasite gp195. BVp42 was additionally shown to produce typical merozoite surface staining patterns as demonstrated by indirect immunofluorescence assays ("IFA"). The IFA titers obtained after the fourth immunization reached levels which exceeded the titer levels obtained by immunization with the purified, parasite gp195 immunogen.

The data and results provided in Example 10 on page 46, lines 1-19, demonstrate that the BVp42 immunogen is recognized by the antisera of various congenic strains of mice (*e.g.*, different H-2 haplotypes on a B10 background) which were immunized with purified, parasite gp195. All seven mice strains assessed were shown to produce anti-gp195 antibodies recognizing epitopes of BVp42, demonstrating that mice strains exhibiting diverse MHC haplotypes are capable of producing antibodies which recognize BVp42. The data and results provided in Example 11 (on page 46, lines 21-33 of the specification) show that anti-BVp42 antibodies react with both homologous (*e.g.*, FUP parasite isolate) and heterologous (*e.g.*, FVO parasite isolate) gp195 antigens as demonstrated by identical ELISA titers and binding curves obtained using anti-BVp42 antibodies with both the homologous and heterologous forms of gp195 immunogen. Similar results were obtained in IFA assays using FVO (*e.g.*, heterologous parasite) and FUP (*e.g.*, homologous parasite) merozoites.

The anti-BVp42 antibodies were additionally demonstrated to strongly or completely inhibit the *in vivo* growth of heterologous (*e.g.*, FVO and Hond-1) and homologous parasites (*e.g.*, FUP and Pf857) in a well-established primate model. The data and results provided in Example 12 (on page 48, lines 1-34 and page 49, line 1) illustrate that *Aotus* monkeys immunized with either QS-21 or ISA51 adjuvant formulations generated antibody responses

to BVp42 immunogen wherein a significant boosting of antibody titer was observed after the second immunization (*see e.g.*, Figs. 8C and 8D, respectively). In addition, all *Aotus* monkeys immunized with either BVp42/QS21 or BVp42/ISA51 continued to develop increased antibody levels with repeated immunization and achieved peak antibody titers after the fourth immunization. Test animal cell mediated immune responses induced by immunization with BVp42 immunogen were assessed by either antigen specific T-cell proliferation assays (*e.g.*, *see* Fig. 9) or by measurement of cytokine producing cells in BVp42 immunogen-stimulated T-cell cultures (*e.g.*, *see* Figs. 10A-B). The cytokine data demonstrate that the claimed BVp42/QS21 and BVp42/ISA51 formulations induce priming of both Th1 and Th2-like lymphocyte populations in immunized *Aotus* monkeys (page 50, lines 15-19).

The data and results provided in Example 13 on page 50, lines 26-28, demonstrate high levels of parasite growth inhibition (*e.g.*, 94.3% and 92.3%) in the test animal immunized with BVp42/ISA51 which displayed the highest level of protective immunity. The data and results provided in Example 15 on page 54, lines 16-21, moreover, demonstrate that the course of infection in three of the four test animals immunized with BVp42/QS21 was significantly reduced relative to control group animals (*see e.g.*, Fig.11B). Test animals were additionally shown to experience prolonged periods of controlled parasite multiplication. Following the parasite multiplication phase, one test animal was even demonstrated to <u>self-cure</u> from infection due to clearance of parasites from the peripheral blood (page 54, lines 19-21). In addition, two of the three test animals immunized with BVp42/ISA51 experienced a prolonged prepatent period (*e.g.*, relative to control animals) after which time parasitemia increased to moderate levels and was then controlled for significant periods (*see* Fig. 11D). For example, the test animal immunized with BVp42/ISA51 displaying the highest level of protective immunity exhibited no detectable parasitemia until 23 days after challenge. Subsequent to this time, parasitemia levels peaked, abruptly dropped to low levels, and then <u>disappeared entirely from the host peripheral circulation</u> (page 54, lines 30-35). One of the test animals immunized with BVp42/ISA51, moreover, remained healthy and vigorous throughout the infection period and exhibited no reduction in erythrocyte count (page 54, line 35; page 55, line 1).

Further, Table 11 (page 55) provides ELISA titers of test animals immunized with BVp42/QS21 or BVp42/ISA51 and challenged with three different *P. falciparum* solid phase immunogens (*e.g.*, recombinant FUP BVp42, recombinant FVO BVp42, and parasite derived FUP MSP1). The data in Table 11 indicate that test animals immunized with BVp42/QS21 produced significantly high titers to both homologous and heterologous immunogen. A high level of cross reactivity was additionally demonstrated between the antibodies produced against recombinant p42 MSP1 and antibodies generated against homologous MSP1 purified from parasite extracts.

Accordingly, one of ordinary skill in the art would conclude that the specification discloses both structural (size, amino acid sequence coordinates relative to gp195 and homology of amino acid sequences) and functional characteristics (immunogenicity, the ability to elicit antibodies that are cross reactive between different gp 195 antigens, and effectiveness in vaccines) that are shared by a representative number of species of p42 polypeptides. Therefore, based on the specification, one of ordinary skill in the art would understand that Applicants invention includes any p42 polypeptide from any *Plasmodium falciparum* isolate, and that these can be readily identified at least as sharing an epitope in common with an epitope of the p42 polypeptides disclosed in SEQ.ID NOS 2-5. Applicant therefore respectfully requests that the Examiner withdraw the rejection of claims 37-55 under 35 U.S.C. §112, first paragraph.

Claim Rejections - 35 USC §112, second Paragraph

Claim 54 was rejected under 35 USC § 112, second paragraph, for reciting "substantially reduces" plasmodium parasitemia in said animal The Examiner was of the opinion that one of ordinary skill in the art would not be able to determine what level of reduction would be necessary achieve "substantial reduction" or even what would be merely average reduction.

Applicant has amended claim 54 to recite in part that the immune response provides at least 92% inhibition of plasmodium parasitemia in said animal. A definition for %

inhibition and support for the 92% limitation as being within the original intent of "substantially reduces" may be found in Example 8, at page 39 and in the data provided in the bottom half of Table 3 on page 40.

The amendment overcomes the rejection for indefiniteness. The Examiner is therefore respectfully requested to withdraw the rejection of claim 54 under 35 USC § 112, second paragraph.

Claim Rejections - 35 USC §102(e) over U.S. Patent No. 6,270,800 to Speaker *et al.*

Claims 37 –47, 50 and 53-55 were rejected as anticipated under 35 USC §102(e) by Speaker *et al.* More specifically, the Examiner argued that the lack of any structural requirements in the claims for what constitutes a p42 polypeptide or fragments and variants thereof necessitated an anticipation rejection based on Speaker *et al* , which discloses vaccines including adjuvants with bovine herpes virus polypeptides expressed in insect cells. Applicant respectfully submits that the present amendment clearly obviates this ground of rejection. Speaker *et al.* plainly has nothing whatsoever to do with *Plasmodium falciparum* polypeptides, let alone with p42 polypeptides derived from the C-terminal processing fragment of the major merozoite surface protein gp195 from a *Plasmodium falciparum* isolate, which polypeptide shares at least one antigenic epitope with a polypeptide according to any one of SEQ.ID NOs. 2-5. The claims as presently amended provide more than adequate structural limitations to distinguish the claimed polypeptides and vaccines from the cited art. Therefore Applicant requests that the Examiner withdraw the rejection of claims 37–47, 50 and 53-55 as anticipated by Speaker *et al*, under § 102(e).

CONCLUSION

Applicant respectfully submits that the Claims are in condition for allowance. If, upon review, the Examiner feels there are additional outstanding issues, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

37. (Amended) A pharmaceutical composition for treating plasmodium parasitemia in a mammal, said composition comprising:

an isolated p42 polypeptide comprising at least a portion of the 42 kDa C-terminal processing fragment of major merozoite surface protein gp195 from a *Plasmodium falciparum* isolate, wherein said isolated p42 polypeptide shares at least one antigenic epitope with a polypeptide according to any one of SEQ.ID NOs. 2-5, in combination with an adjuvant selected from the group consisting of QS-21 and ISA51 and mixtures thereof.

41. (Amended) The pharmaceutical composition of Claim 3[9]7, wherein said isolated p42 polypeptide is a [*Plasmodium falciparum* polypeptide]native sequence p42 polypeptide.

42. (Amended) The pharmaceutical composition of Claim [41] 37, wherein said [*Plasmodium falciparum* polypeptide]p42 polypeptide comprises SEQ. ID No. 8[selected from the group consisting of MAD, K1 and Wellcome].

50. (Amended) An anti-plasmodium vaccine comprising an immunogenic amount of an isolated p42 polypeptide comprising at least a portion of the C-terminal processing fragment of major merozoite surface protein gp195 from a *Plasmodium falciparum* isolate, wherein said isolated p42 polypeptide shares at least one antigenic epitope with a polypeptide according to any one of SEQ.ID NOs. 2-5, said p42 polypeptide being expressed by an insect cell which contains a vector that encodes said polypeptide, in combination with an adjuvant selected from the group consisting of QS21 and ISA51 and mixtures thereof,

wherein said isolated p42 polypeptide is more immunogenic in a mammalian host than is the same polypeptide expressed in yeast.

54. (Amended) The method of Claim 53, wherein said immune response [substantially reduces] provides at least 92% inhibition of plasmodium parasitemia in said mammal.

APPENDIX OF PENDING CLAIMS

37. (Amended) A pharmaceutical composition for treating plasmodium parasitemia in a mammal, said composition comprising:

an isolated p42 polypeptide comprising at least a portion of the 42 kDa C-terminal processing fragment of major merozoite surface protein gp195 from a *Plasmodium falciparum* isolate, wherein said isolated p42 polypeptide shares at least one antigenic epitope with a polypeptide according to any one of SEQ.ID NOs. 2-5, in combination with an adjuvant selected from the group consisting of QS-21 and ISA51 and mixtures thereof.

38. The pharmaceutical composition of Claim 37, further comprising a pharmaceutically acceptable carrier.

39. The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide is expressed by an insect cell which contains a vector that encodes said polypeptide, and wherein said polypeptide is more immunogenic in a mammalian host than is the same polypeptide expressed in yeast.

40. The pharmaceutical composition of Claim 39, wherein said insect cell is selected from the group consisting of *Spodoptera frugiperda*, *Spodoptera exiaua*, *Choristoneura fumiferana*, *Trichoplusia ni* and *Spodoptera littoralis*.

41. (Amended) The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide is a native sequence p42 polypeptide.

42. (Amended) The pharmaceutical composition of Claim 37, wherein said p42 polypeptide comprises SEQ. ID No. 8.

43. The pharmaceutical composition of Claim 37, wherein the transmembrane domain of said isolated p42 polypeptide is deleted.

44. The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide is fused to a second polypeptide.

45. The pharmaceutical composition of Claim 44, wherein said second polypeptide is a leader sequence fused to the amino terminus of said isolated p42 polypeptide.

46. The pharmaceutical composition of Claim 39, wherein said vector is a baculovirus

vector.

47. The pharmaceutical composition of Claim 39, wherein said mammalian host is a primate.

48. The pharmaceutical composition of Claim 37, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:
(a) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

49. The pharmaceutical composition of Claim 48, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:
(a) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

50. (Amended) An anti-plasmodium vaccine comprising an immunogenic amount of an isolated p42 polypeptide comprising at least a portion of the C-terminal processing fragment of major merozoite surface protein gp195 from a *Plasmodium falciparum* isolate, wherein said isolated p42 polypeptide shares at least one antigenic epitope with a polypeptide according to any one of SEQ.ID NOs. 2-5, said p42 polypeptide being expressed by an insect cell which contains a vector that encodes said polypeptide, in combination with an adjuvant selected from the group consisting of QS21 and ISA51 and mixtures thereof,

wherein said isolated p42 polypeptide is more immunogenic in a mammalian host than is the same polypeptide expressed in yeast.

51. The vaccine of Claim 50, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:
(a) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 394 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 377 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

52. The vaccine of Claim 51, wherein said isolated p42 polypeptide comprises an amino acid sequence selected from the group consisting of:

(a) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:2;
(b) amino acids 1 to 373 of the amino acid sequence of SEQ ID NO:3;
(c) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:4;
(d) amino acids 1 to 356 of the amino acid sequence of SEQ ID NO:5; and
(e) combinations thereof.

53. A method of inducing an anti-plasmodium immune response in a mammal comprising administering to said mammal the vaccine of Claims 50, 51 or 52.

54. (Amended) The method of Claim 53, wherein said immune response provides at least 92% inhibition of plasmodium parasitemia in said mammal.

55. The method of Claim 53, wherein said mammal is a primate.